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Bcl-x Is Not Required for Maintenance of Follicles and Corpus Luteum in the Postnatal Mouse Ovary[1]

Gregory Riedlinger,[3] Ryugo Okagaki,[3] Kay-Uwe Wagner,[3,4] Edmund B. Rucker III,[3,5] Takami Oka,[3] Keiko Miyoshi,[3] Jodi A. Flaws,[6] and Lothar Hennighausen[2,3]

Laboratory of Genetics and Physiology,[3] National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892
Eppley Institute for Research in Cancer and Allied Diseases,[4] University of Nebraska Medical Center, Omaha, Nebraska 68198
Animal Sciences Unit,[5] University of Missouri, Columbia, Missouri 65211
Department of Epidemiology and Preventive Medicine,[6] University of Maryland Medical School, Baltimore, Maryland 21201

ABSTRACT

It has been proposed that Bcl-x is a key survival factor in many cell types, and that the *bcl-x* gene is activated by the transcription factor Stat5 through cytokine signals. In support of this, it has been demonstrated that the survival of mouse primordial germ cells during embryogenesis depends on the presence of Bcl-x. We have now investigated whether, in the mouse, Bcl-x is required for the postnatal maintenance of follicles and luteal cells, and whether Stat5 activates the *bcl-x* gene. The *bcl-x* gene was deleted in these cells within the mouse using Cre-loxP recombination. Loss of the *bcl-x* gene did not affect the numbers of primordial, primary, and antral follicles. Furthermore, expression of the *bcl-x* gene in the ovary was independent of Stat5 and its activating hormone, prolactin. To determine whether the prolactin receptor (PrlR), Stat5, and Bcl-x were required for establishment and maintenance of the corpus luteum, we induced pseudopregnancies in the respective gene-deletion mice. Whereas luteal cells underwent apoptosis in the absence of the PrlR, no changes were observed in the absence of Stat5 or Bcl-x.

apoptosis, corpus luteum, follicle, granulosa cells

INTRODUCTION

Postnatal follicular atresia and luteolysis have been linked to apoptosis [1], but the molecular mechanisms are not known. Pro- and antiapoptotic members of the Bcl-2 family have been localized in oocytes, granulosa cells, and luteal cells, suggesting that they control survival and atresia of the respective cell types. Bcl-x is the most abundant antiapoptotic member of the Bcl-2 family, and it has been found in hen granulosa cells [2, 3]. Bcl-x levels were highest in granulosa cells of preovulatory follicles, which correlated with their resistance to apoptotic stimuli in vitro. Bcl-x has also been detected in oocytes and granulosa cells in the mouse [4] and human [5]. Genetic experiments have demonstrated that the survival of primordial germ cells during embryogenesis depends on the presence of Bcl-x [6]. Thus, we hypothesized that Bcl-x is also required for the maintenance of follicles and granulosa cells.

Deletion of the *bcl-x* gene from the mouse results in lethality at approximately Embryonic Day 12.5 (E12.5) [7]. Thus, it is not possible to establish the role of Bcl-x in the ontogeny of ovaries in the postnatal mouse. To test whether Bcl-x is required for the maintenance of follicles, granulosa and luteal cells, and oocytes, we have inactivated the *bcl-x* gene in these cells using Cre-loxP-based recombination. We have generated mice that carry loxP sites in the promoter and second intron of the *bcl-x* gene [6] as well as mice that express the Cre recombinase in several cell types in the ovary [8].

In addition, we have used the conditional Bcl-x-null mice to test the hypothesis that *bcl-x* gene transcription in the ovary is activated by prolactin (Prl) through Stat5. Prolactin is released during estrus and is thought to be obligatory for the maintenance of luteal function. Previous studies have shown that Prl receptor (PrlR) levels increase when granulosa cells differentiate, and that PrlR levels are highest in luteal cells [9]. Previous studies have also shown that PrlR-mediated activation of Stat5 coincides with luteinization [9], and that transcription of the *bcl-x* gene can be activated by Stat5 in some cell lines [10, 11]. To our knowledge, however, no in vivo studies have directly tested whether *bcl-x* gene transcription in the ovary is activated by Prl through Stat5. Thus, we used mice deficient in Bcl-x, PrlR, and Stat5 to test this hypothesis.

MATERIALS AND METHODS

Animals

Mice were housed in a 12L:12D photoperiod at 22°C and 80% relative humidity with food and water ad libitum. All animal procedures were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committees of the University of Maryland and the NIH. These animals were covered under the NIH protocol K990631.

Transgenic and Gene Knock-Out Mice

Generation of mice carrying an MMTV-Cre transgene and of mice carrying a *bcl-x* gene with loxP sequences (floxed gene) has been reported previously [6, 8]. Polymerase chain reaction (PCR) was used to distinguish the floxed *bcl-x* gene from the wild-type allele using primers 5′-CGGTTG-CCTAGCAACGGGGC-3′ and 5′-CTCCCACAGTGGAGACCTCG-3′. Reaction conditions were 4 min at 96°C (1 cycle); 45 sec at 96°C, 45 sec at 58°C, and 1 min at 72°C (30 cycles); and 5 min at 72°C (1 cycle). The PCR products were 300 base pairs (bp) for the floxed allele and 200 bp for the wild-type allele. The presence of the Cre-mediated recombined *bcl-x* allele (resulting in a *bcl-x*-null allele) was detected using primers 5′-

[1]Supported in part by NIH R01 HD 38955 (J.F.).
[2]Correspondence: Lothar Hennighausen, Laboratory of Genetics and Physiology, National Institutes of Health, Bldg. 8, Room 101, 8 Center Drive, Bethesda, MD 20892. FAX: 301 480 7312; e-mail: hennighausen@nih.gov

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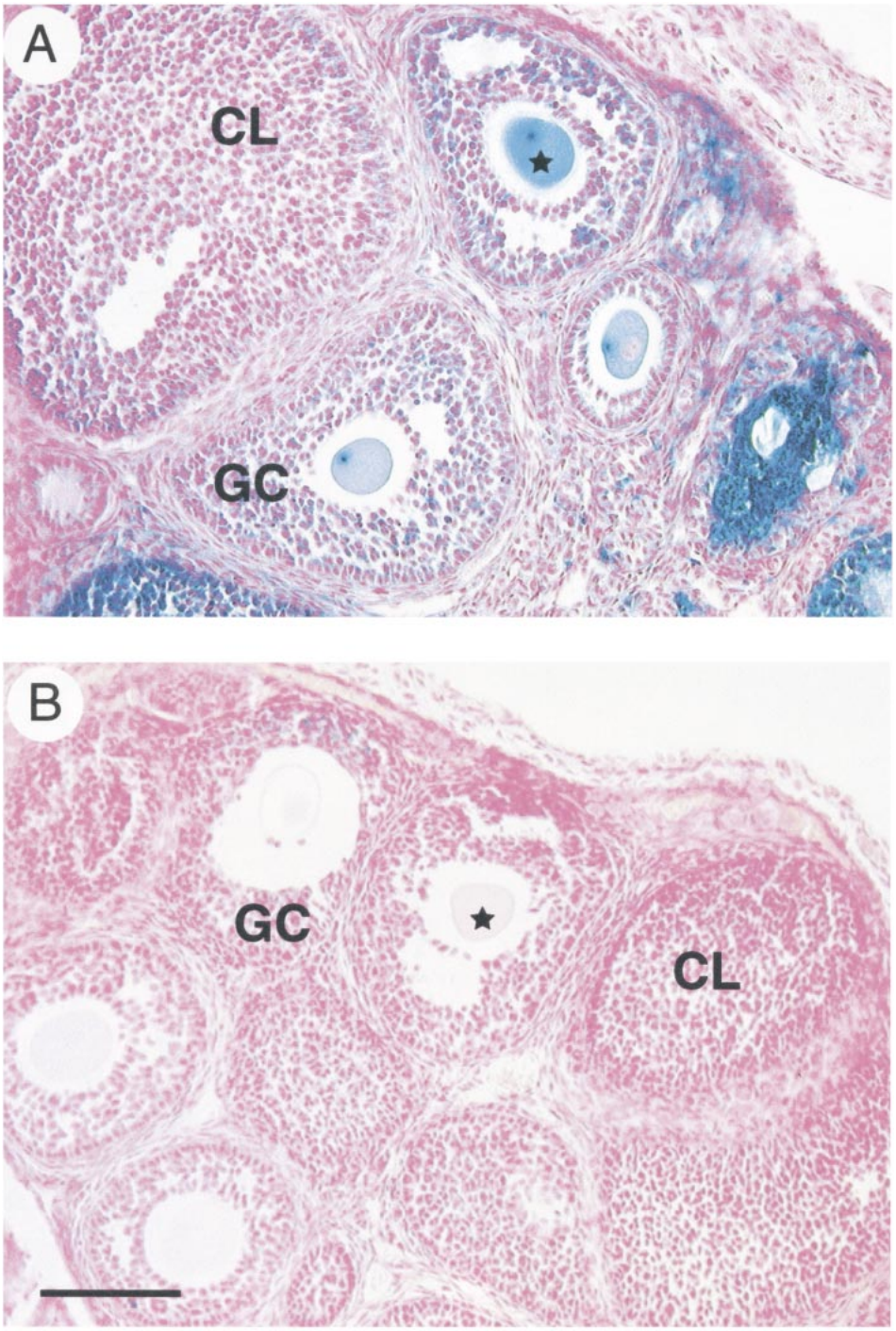

FIG. 1. Cre-mediated recombination in ovaries. β-Galactosidase activity was analyzed in ovaries from 6-wk-old virgin females carrying the MMTV-Cre transgene, two floxed bcl-x alleles, and the *Rosa26* locus (**A**). As a control, ovaries from bcl-x fl/fl mice were used (**B**). The counterstain was Nuclear Fast Red. Note that the blue stain of the oocytes is the result of Cre-activated recombination of the *Rosa26* locus. The star indicates an oocyte. GC, Granulosa cells; CL, corpus luteum. Bar = 0.1 mm.

AATGGCCAGTACTAGTGAACC-3′ and 5′-TCAGAAGCCGCAATATCCCC-3′ with the same cycling conditions as described above. The *bcl-x*-null allele was presented by a PCR product of 150 bp. A PCR method to genotype the LacZ reporter in the targeted *Rosa26* locus has been published previously [12].

The PrlR-null [13] and Stat5-null [14] mice were obtained from Drs. Paul Kelly (Paris, France) and Jim Ihle (Memphis, TN), respectively. In the Stat5-null mice, both the *Stat5a* and *5b* genes had been inactivated. The Stat5- and PrlR-null mice were used for superovulation studies, Western blot analysis of ovaries, histological analysis of ovaries, and TUNEL assay of ovaries from pseudopregnant mice. These mice were infertile and not bred.

PCR assays were used to determine the time during mouse development at which Cre-mediated *bcl-x* gene inactivation and deletion in granulosa cells occurred. Four-week-old mice that carried two floxed bcl-x alleles and the MMTV-Cre (A) transgene (referred to as bcl-x fl/fl; Cre) as well as bcl-x fl/+ mice were injected with 5 IU of eCG i.p. Ovaries from three bcl-x fl/fl; Cre and three bcl-x fl/+ mice were harvested 48 h later. Large antral follicles were punctured with a needle to collect granulosa cells. Between 50 and 100 granulosa cells were lysed in a solution consisting of 1% (v/v) NP-40, 6 mM dithiothreitol, and 10 U/μl of RNase inhibitor (Promega, Madison, WI). The PCR assays were performed using 1 μl of lysed granulosa cells for the floxed and recombined Bcl-x allele as described above. Whole ovaries were collected from mice on E15.5, as determined by plug formation, and PCR was performed on isolated DNA for the presence of the recombined *bcl-x* allele. Ovaries from four bcl-x fl/fl; Cre and three bcl-x fl/fl fetuses were analyzed. Embryonic Day 15.5 was chosen because the MMTV-LTR is activated during that time [15].

Superovulation

To determine any defect in ovulation, mice were superovulated by i.p. injection of 5 IU of eCG followed 48 h later by i.p. injection of 5 IU of

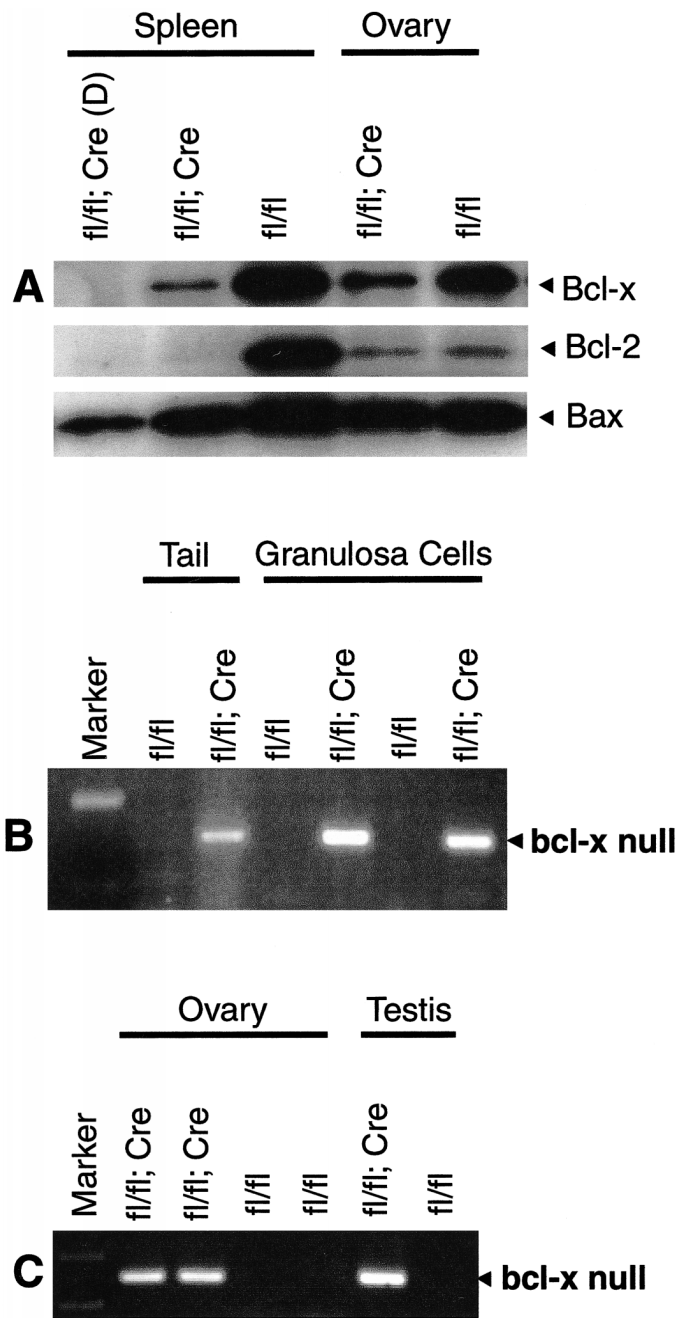

FIG. 2. **A**) Western blot analysis of spleen and ovarian tissue from 4-wk-old bcl-x fl/fl; Cre mice and bcl-x fl/fl mice without the Cre transgene. Protein from the spleen of a bcl-x fl/fl; Cre (D) female was used as a control. This mouse had undergone more than 95% recombination [12]. Four different bcl-x fl/fl and bcl-x fl/fl; Cre mouse ovaries each were analyzed for Bcl-x. Three different bcl-x fl/fl and bcl-x fl/fl; Cre mouse ovaries each were analyzed for Bcl-2 and Bax. **B**) PCR analysis of the recombined *bcl-x* allele in isolated granulosa cells. Granulosa cells from 4-wk-old bcl-x fl/+ and bcl-x fl/+; Cre mice were isolated, and the presence of the recombined allele was monitored. Ovaries from three bcl-x fl/+ and three bcl-x fl/+; Cre mice were harvested. Tail DNA from a bcl-x fl/fl; Cre female served as a positive control. **C**) PCR analysis of the recombined *bcl-x* allele in testes and ovaries from E15.5 embryos. Ovaries from four bcl-x fl/fl; Cre and three bcl-x fl/fl fetuses were analyzed.

hCG. Twenty-four hours after hCG injection, ovaries and oviducts were collected, and oviducts were punctured with a needle to release oocyte and cumulus cells. Released oocytes were then counted. Three bcl-x fl/fl, three bcl-x fl/fl; Cre, four Stat5 −/−, four Stat5 +/−, four PrlR −/−, and three wild-type mice were used in these experiments. Data are presented as mean ± SEM.

Histological Evaluation of Ovaries

Ovaries were fixed in Bouin solution overnight and then washed in 70% ethanol. Ovaries were embedded in paraplast (VWR Scientific, Buffalo Grove, IL) and then serial sectioned (thickness, 8 μm) through the entire tissue, mounted on glass slides, and stained with Weigert hematoxylin-picric acid methylene blue. Every 10th section was analyzed for primordial, primary, and preantral/antral follicle numbers. Three virgin mice each were used for the 5-wk bcl-x fl/fl; Cre and bcl-x fl/fl as well as for the 3-mo bcl-x fl/fl time points. Six virgin mice were used for the 3-mo bcl-x fl/fl; Cre time point. All data are presented as mean ± SEM. The X-Gal staining technique has been reported previously [8].

Tissues were fixed in 10% buffered formalin overnight for Bcl-x immunohistochemistry. The Bcl-x antibody used for staining (SC-634; Santa Cruz Biotechnology, Santa Cruz, CA) was used at a 1:100 (v/v) dilution. Biotinylated secondary antibodies were detected using Vectastain Elite ABC kit (Vector, Burlingame, CA). The slides were counterstained with hematoxylin.

To identify nuclei with DNA strand breaks, TUNEL was performed using the Apoptag protocol (Intergen, Purchase, NY). Slides were counterstained with 0.1% methyl green for 1 min. Pseudopregnancy was induced in mature PrlR-null, Stat5-null, bcl-x fl/fl; Cre, and bcl-x fl/fl virgin mice (i.p. injection of 5 IU of eCG followed 48 h later by i.p. injection of 5 IU of hCG every 24 h for 5–7 days). At least three mice of each genotype were analyzed.

Western Blot Analysis

Tissues were homogenized in ice-cold lysis buffer consisting of 40 mM Tris-HCl (pH 8.0), 280 mM NaCl, 20% glycerol, 1% NP-40, 4 mM EDTA, 20 mM NaF, 100 μg/ml of PMSF, 40 μg/ml of aprotinin, 40 μg/ml of leupeptin, and 2 mM Na-vanadate. Protein concentration was determined using the BCA Protein Assay Kit (Pierce, Rockford, IL) with BSA as a standard. Forty micrograms of protein were loaded per lane, separated by SDS-PAGE on a 16% gel, and transferred to a polyvinylidene difluoride membrane (Invitrogen, Carlsbad, CA). The membranes were blocked overnight at 4°C with TBST (50 mM Tris-HCl [pH 7.5], 0.15 M NaCl, and 0.05% Tween-20) containing 3% nonfat dry milk. They were incubated for 1 h with rabbit anti-Bcl-x (SC-634), anti-Bax (SC-493; Santa Cruz Biotechnology), anti-Bcl-2 (no. 06-474; Upstate Biotechnology, Lake Placid, NY), anti-Stat5a, or anti-Stat5b serum [16], diluted at 1:500, 1:500, 1:500, 1:20 000, or 1:10 000, respectively, with 3% nonfat dry milk in TBST. Horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G was used as the secondary antibody and reacted at 1:5000 with 3% nonfat dry milk in TBST for 30 min. Bands were visualized using SuperSignal West Pico Chemiluminescent Substrate (Pierce). Protein was collected from pseudopregnant ovaries (i.p. injection of 5 IU of eCG followed 48 h later by i.p. injection of 5 IU of hCG every 24 h for 5–7 days) from three PrlR-null, PrlR +/−, wild-type, Stat5-null, and Stat5 +/− mature mice each for Western blot analysis. Protein was also collected from four 4-wk-old virgin bcl-x fl/fl; Cre and four bcl-x fl/fl mice.

Progesterone Levels

Progesterone levels were measured by RIA using Coat-A-Count (Diagnostic Products Corporation, Los Angeles, CA). Mice were anesthetized, and blood was collected by phlebotomy from the retro-orbital plexus. Serum was separated from cells by centrifugation and stored at −80°C. Serum from three bcl-x fl/fl; Cre and three bcl-x fl/fl mice was analyzed. Data are presented as mean ± SEM.

Statistical Analysis

The mean number of ovarian follicles was calculated using ovaries from at least three different animals. The mean number of oocytes recovered following superovulation was determined using at least three different mice for each genotype. The mean progesterone levels were calculated using three different animals. Differences in follicle numbers, recovered oocytes, and progesterone levels were evaluated by a *t*-test for equality of means, with statistical significance being assigned at $P < 0.05$.

RESULTS

Deletion of the bcl-x *Gene in Oocytes, Granulosa Cells, and Luteal Cells*

Because Bcl-x-null mice die at approximately E12.5, we deleted the *bcl-x* gene in distinct cell types using a Cre-

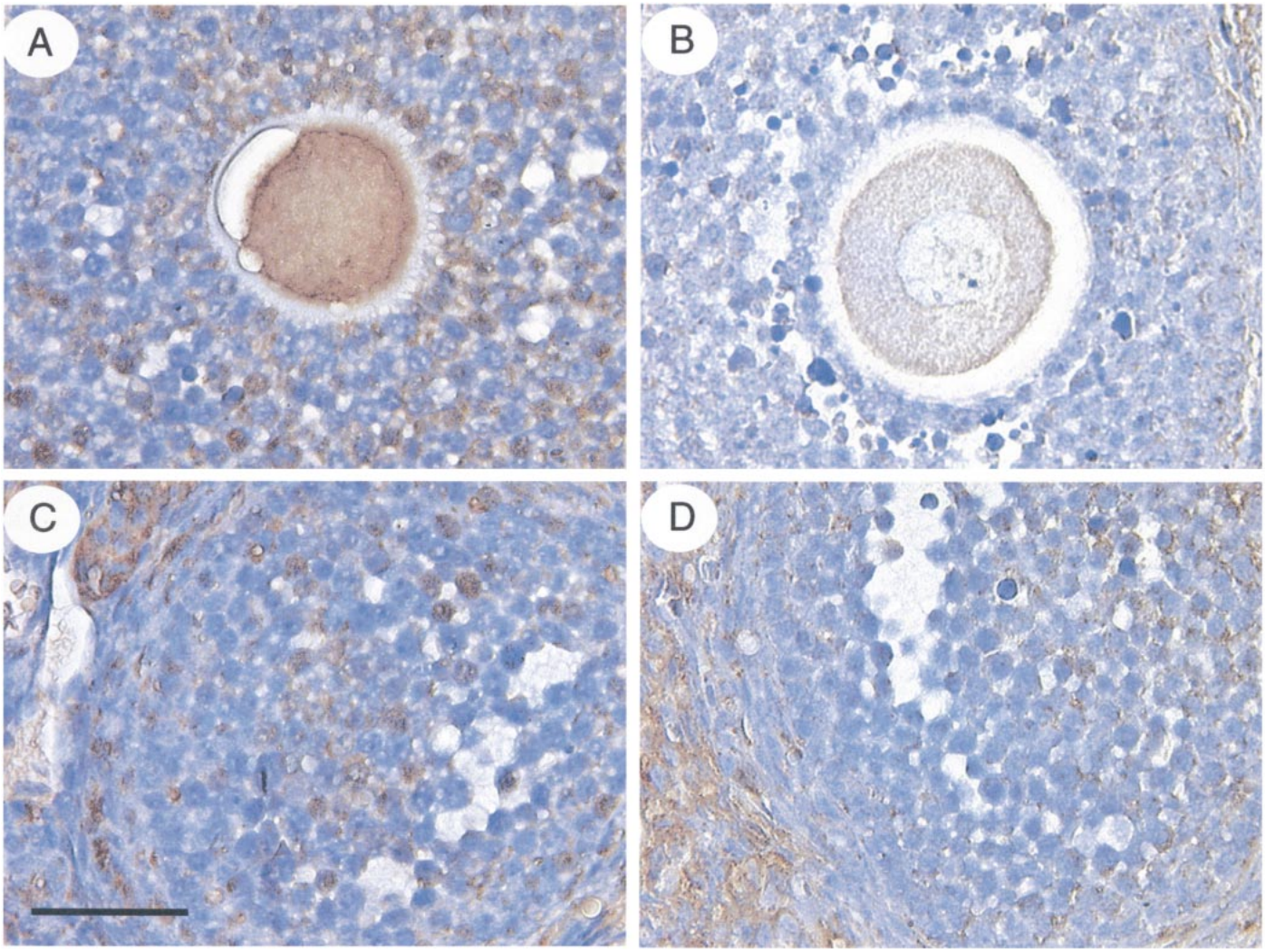

FIG. 3. Immunohistochemical analysis of Bcl-x in ovaries from bcl-x fl/fl (**A** and **C**) and bcl-x fl/fl; Cre (**B** and **D**) mice. Bcl-x was identified in oocytes and granulosa cells from bcl-x fl/fl (**A**) but not bcl-x fl/fl; Cre (**B**) mice. Whereas Bcl-x was also identified in luteal cells from bcl-x fl/fl (**C**), its levels in bcl-x fl/fl; Cre (**D**) mice were sharply reduced. Bar = 0.05 mm.

loxP recombination approach. One line of transgenic mice that expresses Cre recombinase under control of the MMTV-LTR exhibits Cre activity in the ovary [8]. We have now used in situ and PCR assays to establish the cell types within the ovary that express Cre. The *Rosa26* reporter allele was introduced into MMTV-Cre mice, and the ovaries were analyzed. Blue-stained cells indicated that Cre-mediated recombination had been accomplished. Recombination was observed in oocytes, granulosa cells, and corpora lutea (CL) (Fig. 1A).

To delete the *bcl-x* gene in these cells, we generated females that carry two floxed *bcl-x* alleles (fl/fl) and the MMTV-Cre transgene (referred to as bcl-x fl/fl; Cre mice, as previously defined). These mice were overtly normal but exhibited reduced fertility. Most females that carried two floxed alleles but no Cre transgene conceived within one estrous cycle and gave birth to normal-sized litters, but only approximately 30% of the bcl-x fl/fl; Cre females ($n > 10$) gave birth to small litters (six pups or less). Invariably, the offspring carried *bcl-x*-null alleles (data not shown), establishing that Bcl-x was not required for oocyte survival. Western blot analyses from ovaries and spleen tissue of bcl-x fl/fl; Cre females showed reduced levels of Bcl-x compared to control mice (Fig. 2A). To evaluate whether the reduction of Bcl-x resulted in altered levels of other Bcl-2 family members, we determined the levels of cell survival factor Bcl-2 and of cell death factor Bax. Levels of Bcl-2 and Bax were maintained in bcl-x fl/fl; Cre ovaries compared to control littermates (Fig. 2A). The absence of Bcl-2 in spleen from Bcl-x-null mice reflects the loss of T and B cells as well as of mature erythrocytes, in which Bcl-2 is highly expressed [12]. Furthermore, we performed PCR analyses on isolated granulosa cells and detected the *bcl-x*-null allele (Fig. 2B), demonstrating that the *bcl-x* gene was deleted in this cell type. Deletion of the *bcl-x* gene in ovaries was detected as early as E15.5 (Fig. 2C), a time when the MMTV-Cre transgene is active.

Because ovaries from bcl-x fl/fl; Cre mice still contained residual Bcl-x levels (Fig. 2A), we determined by immunohistochemistry the cell types that contain abundant Bcl-x (Fig. 3). Bcl-x was detected in oocytes and many granulosa and luteal cells from bcl-x fl/fl mice. On deletion of the *bcl-x* gene in bcl-x fl/fl; Cre mice, Bcl-x levels were sharply reduced in these cell types (Fig. 3). Thus, the loss of Bcl-x expression is seen in those cells that expressed the *Cre* gene based on the *Rosa* reporter gene (Fig. 1).

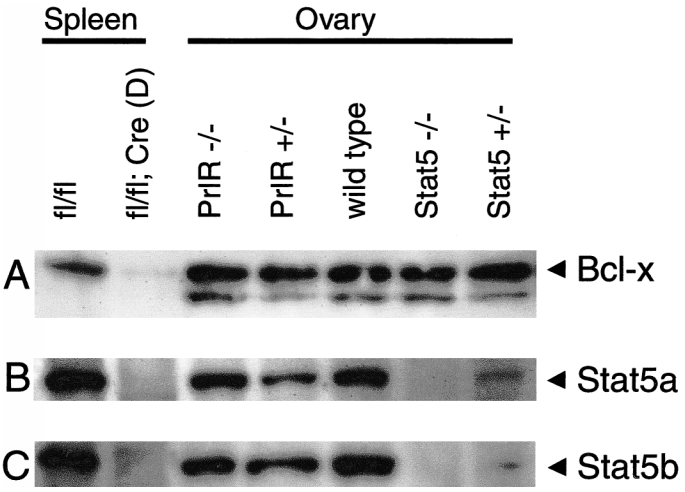

FIG. 4. Analyses of Bcl-x (**A**), Stat5a (**B**), and Stat5b (**C**) in ovarian tissue. Total protein from spleens and ovaries was separated in a 12% gel, blotted onto a membrane, and probed for Bcl-x (**A**), Stat5a (**B**), and Stat5b (**C**). Total spleen protein from bcl-x fl/fl and bcl-x fl/fl; Cre (D) mice was analyzed. Ovary protein was analyzed from wild-type, PrlR +/−, PrlR −/−, Stat5 +/−, and Stat5 −/− pseudopregnant mice (i.p. injection of 5 IU of eCG followed 48 h later by injection of 5 IU of hCG every 24 h for 5–7 days). Three mice of each genotype were analyzed.

Maintenance of Follicle Numbers in Postnatal Life in the Absence of Bcl-x

Bcl-x is required for the survival of primordial germ cells [6]. To evaluate whether Bcl-x is required for maintaining the postnatal pool of follicles, we performed morphometric analyses on ovaries from bcl-x fl/fl and bcl-x fl/fl; Cre females. At 5 wk and 3 mo of age, no differences were observed for primordial, primary, and antral follicle numbers in bcl-x fl/fl; Cre ovaries compared with bcl-x fl/fl control littermates (Table 1). Bcl-x fl/fl; Cre ovaries were also healthy in appearance and morphologically similar to ovaries of control littermates. For 5 wk, n = 3 for bcl-x fl/fl; Cre and bcl-x fl/fl mice, $P = 0.184$ for primordial follicles, $P = 0.305$ for primary follicles, and $P = 0.989$ for antral follicles. For 3 mo, n = 6 for bcl-x fl/fl; Cre mice and n = 3 for bcl-x fl/fl mice, $P = 0.156$ for primordial follicles, $P = 0.357$ for primary follicles, and $P = 0.206$ for antral follicles.

Ovulation and Progesterone Levels in the Absence of Bcl-x

To evaluate whether the reduced fertility of the bcl-x fl/fl; Cre females resulted from failure to ovulate, we superovulated mice and determined the number of oocytes. Both bcl-x fl/fl (14 ± 3.5) and bcl-x fl/fl; Cre (19 ± 5, $P = 0.459$) females had similar numbers of oocytes recovered in their oviducts. We also superovulated Stat5- and PrlR-null mice and determined the number of oocytes. Stat5 −/− mice had 37 ± 9 and Stat5 +/− mice 41 ± 19 ($P = 0.858$) oocytes recovered from their oviducts. The PrlR −/− mice had 10 ± 1 and wild-type mice 22 ± 6.7 ($P = 0.086$) oocytes recovered from their oviducts. Bcl-x fl/fl; Cre females had a normal estrous cycle, and progesterone levels in pseudopregnant bcl-x fl/fl; Cre mice (30 ± 12 ng/ml) were comparable to those in bcl-x fl/fl control mice (19 ± 7 ng/ml, $P = 0.450$).

TABLE 1. Follicle counts of 5-wk- and 3-mo-old bcl-x fl/fl; Cre and bcl-x fl/fl; Cre mice.

Genotype	Age	Primordial	Primary	Preantral/antral
fl/fl; Cre	5 wk	11 000 ± 1800	3800 ± 1200	4400 ± 1600
fl/fl	5 wk	17 000 ± 3100	2300 ± 460	4400 ± 860
fl/fl; Cre	3 mo	5200 ± 1200	2400 ± 490	4000 ± 590
fl/fl	3 mo	8700 ± 2000	3100 ± 550	5200 ± 270

Expression of the bcl-x *Gene in Ovaries Is Not Controlled by Stat5*

To determine whether expression of the *bcl-x* gene in ovaries is dependent on the PrlR-Stat5 pathway, we analyzed Bcl-x levels in ovaries from mature PrlR- and Stat5-null mice. Similar levels of Bcl-x were found in the presence and absence of the PrlR and Stat5 (Fig. 1A). The specificity of the Bcl-x signal was established with spleens from mice in which the *bcl-x* gene had been deleted in the erythroid compartment [12]. The strong signal observed in spleen from bcl-x fl/fl mice was lost in bcl-x fl/fl; Cre mice (Fig. 4A). The presence of Stat5a and Stat5b in ovarian tissue was confirmed by Western blot analysis (Fig. 4, B and C, respectively). As expected, no Stat5 was detected in Stat5-null mice. The absence of Stat5 in spleen from Bcl-x-null mice reflects the loss of T and B cells as well as of mature erythrocytes from this organ [12].

Maintenance of CL Depends on PrlR But Not Stat5 and Bcl-x

The PrlR- [13] and Stat5-null [14] mice are infertile, possibly as the result of nonfunctional CL. Whereas CL of normal size and appearance were observed in PrlR-null females [13], only a few small CL were reported in Stat5-null mice [14]. We have now evaluated whether PrlR, Stat5, and Bcl-x are required for establishment and maintenance of the CL using the respective gene-deletion mice. Histological analyses demonstrated the presence of CL in mature PrlR- and Stat5-null virgins and in mice from which the *bcl-x* gene had been deleted in granulosa and luteal cells (Fig. 5, A, D, and G, respectively). Because the loss of PrlR, Stat5, and Bcl-x results in severely reduced fertility, it was not possible to investigate pregnancy-mediated maintenance of the CL. Pseudopregnancies were induced with eCG-hCG, and the ovaries were examined after 1 wk. The PrlR-null mice developed hemorrhagic CL (Fig. 5B) and exhibited extensive apoptosis as detected by TUNEL assay (Fig. 5C). In contrast, no overt changes in the CL were observed in the absence of Stat5 (Fig. 5, E and F) and Bcl-x (Fig. 5, H and I). Similarly, the CL were maintained in wild-type mice (Fig. 5, K and L). Whereas we observed CL of regular size in the Stat5-null mice, others have described Stat5-null mice containing no, or only small, CL [14]. The reason for this difference is not clear.

DISCUSSION

Postnatal follicular atresia and luteolysis have been linked to apoptosis, but the underlying molecular mechanisms are not understood. High levels of Bcl-x are found in follicles and preovulatory granulosa cells that are resistant to apoptotic stimuli, suggesting a role in folliculogenesis and luteinization [3]. Because the survival of primordial germ cells during embryogenesis depends on the presence of Bcl-x [6], we hypothesized that this antiapoptotic protein may also be required in the postnatal ovary. We

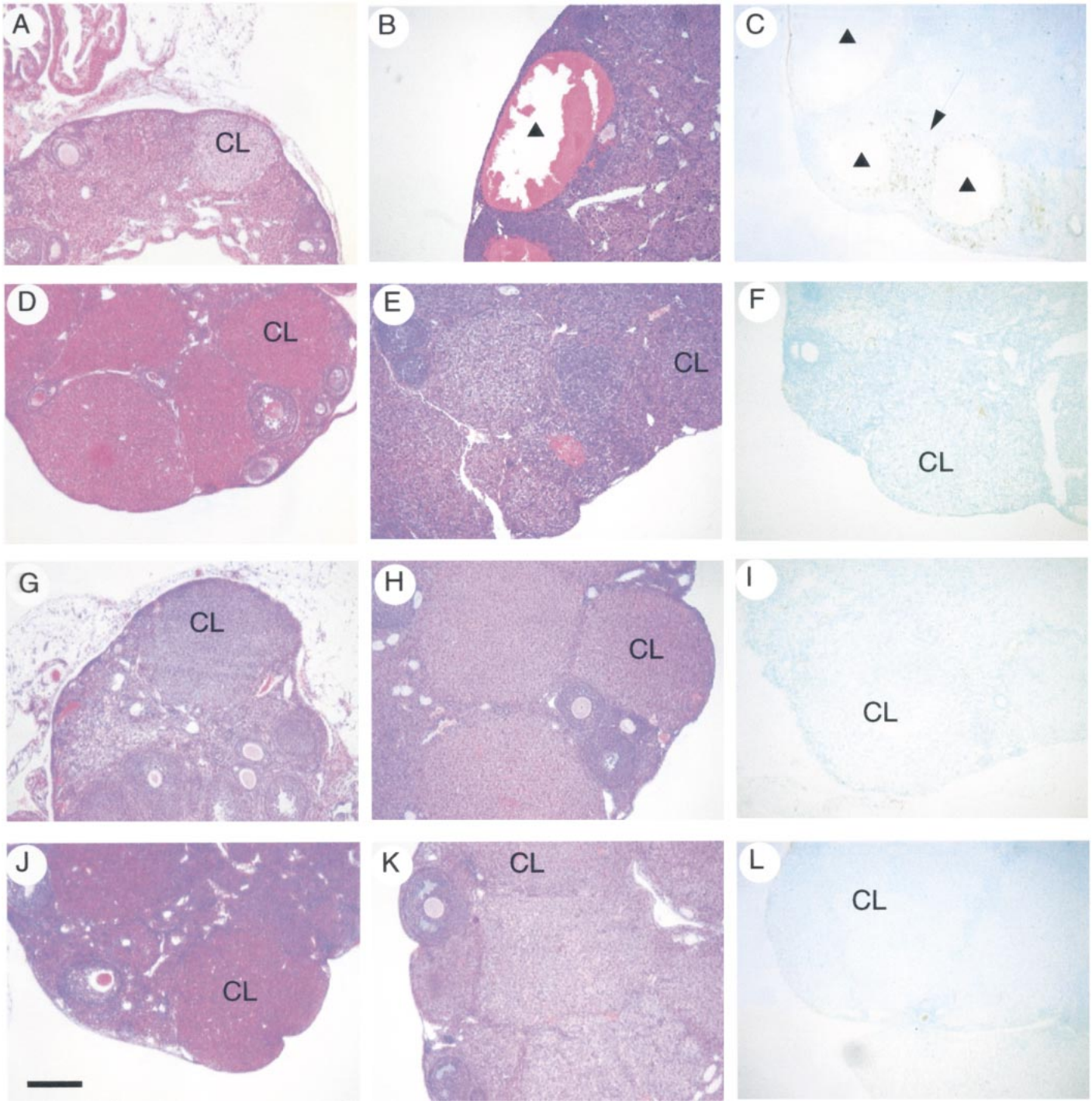

FIG. 5. Histological analysis of ovaries from PrlR-null (**A–C**), Stat5-null (**D–F**), bcl-x fl/fl; Cre (**G–I**), and bcl-x fl/fl control mice (**J–L**). Histological analyses demonstrated the presence of CL in mature PrlR-null virgins (**A**), Stat5-null virgins (**D**), bcl-x fl/fl; Cre virgins (**G**), and bcl-x fl/fl virgins (**J**). The PrlR-null ovaries developed hemorrhagic CL (**B**) and had extensive apoptosis (**C**) as demonstrated by TUNEL assay after 1 wk of pseudopregnancy. Stat5-null (**E** and **F**), bcl-x fl/fl; Cre (**H** and **I**), and bcl-x fl/fl (**K** and **L**) ovaries did not develop hemorrhagic CL and did not exhibit significant levels of apoptosis. Hematoxylin-and-eosin staining and TUNEL assay were performed on three mice of each genotype. CL, Corpus luteum; triangles, hemorrhagic CL; arrowhead, extensive apoptosis. Bar = 0.2 mm.

have used experimental mouse genetics to directly test this hypothesis. Because Bcl-x-null mice die in utero, we have used the Cre-loxP recombination system to delete the *bcl-x* gene from follicles, granulosa cells, and luteal cells. Overall Bcl-x levels in ovarian tissue were reduced to approximately 20–30%, and the loss of Bcl-x in granulosa and luteal cells was confirmed by immunohistochemistry. These mice contained normal numbers of primordial, primary, and antral follicles, and they developed CL producing normal levels of progesterone. Thus, Bcl-x is not required for ovarian development.

Although Bcl-x is the most abundant member of the Bcl-2 family, other antiapoptotic proteins might compensate for the loss of Bcl-x. It could be hypothesized that Bcl-2 compensates for the loss of Bcl-x, but such compensation has not been observed in the fetal gonad [6] or in erythroid

cells [12]. In addition, we did not detect increased levels of Bcl-2 in the Bcl-x-null ovaries, suggesting the absence of compensation through Bcl-2.

Evidence from the hematopoietic system suggests that cytokines can activate *bcl-x* gene transcription through the transcription factor Stat5. In erythroid cells, *bcl-x* gene expression is activated by erythropoietin through the Jak2/Stat5 pathway [17]. We have now shown that Bcl-x levels in ovaries are unaltered in the absence of PrlR or Stat5, suggesting that this pathway does not control transcription of the *bcl-x* gene. However, this does not exclude that *bcl-x* gene expression in the ovary can be induced under certain circumstances through the PrlR-Stat5 axis.

Corpora lutea cannot be maintained in the absence of PrlR, and after 1 wk of pseudopregnancy, most luteal cells have undergone apoptosis. In contrast, no significant loss of luteal cells is observed in the absence of Stat5 and Bcl-x. This demonstrates that the Prl-induced cell survival signal is not mediated by the Jak2/Stat5/Bcl-x pathway. Because cytokine-induced cell survival of other cell types is mediated by Stat5, we suggest that luteal cells are unique and exploit other, yet-to-be-defined cell survival pathways.

NOTE ADDED IN PROOF

We have recently discovered the presence of N-terminally truncated Stat5a and Stat5b in various tissues in Stat5-null mice, including the ovary. These truncated proteins are expressed at levels comparable to wild-type Stat5 protein in the ovary. Data generated from these mice may need to be reevaluated if it is determined that the truncated protein has biological activity.

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